

Finally, study endpoints need to be defined as objective criteria which, if met, would necessitate the removal of the animal from the study. For instance, what level of weight loss would require an animal to be removed? Would dehydration be addressed by treatment with parenteral fluids or by removal of the animal from the study? Also important is the information on who will be monitoring the animals—assessment of pain and distress in rabbits would be difficult because they tend to conceal outward signs of pain and stress. Behavioral changes may be so subtle that they are detectable only after careful observation by trained individuals. Euthanasia methods must also be stated and conform to the 2000 Report of the AVMA Panel on Euthanasia⁶.

1. Animal Welfare Act (7 USC, 2143(a)(3)(C)).
2. Public Health Service. *Policy on Humane Care and Use of Laboratory Animals* IV.C.1.b (US Department of Health and Human Services, Washington, DC, 1986; reprinted 2002).
3. Animal Welfare Act (7 USC, 2143(a)(3)(B)).
4. 9 CFR, 2.31(d)(1)(ix).
5. Institute for Laboratory Animal Research, National Research Council. *Guide for the Care and Use of Laboratory Animals* 60–64 (National Academy Press, Washington, DC, 1996).
6. Beaver, B.V. et al. 2000 Report of the AVMA Panel on Euthanasia. *J. Am. Vet. Med. Assoc.* **218**(5), 669–696 (2001).

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RESPONSE

Broader, and more specific

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The IACUC must evaluate both phases of the proposed procedure: the induction of disease and the investigation of potential therapies. Concerns regarding variability in the development of cancer in this model over the anticipated year-long period need to be addressed, as an early onset could result in pain and distress. If advanced disease states become apparent, the IACUC needs to be informed about what steps are planned to respond to the symptoms. If an animal begins to suffer from signs of cancer metastases, will the surgical procedure be performed earlier than planned or will the

rabbit be euthanized (in essence wasting an animal)? Additionally, very little information is provided on either the toxic effects of diethylnitrosamine in this model or the frequency of post-treatment observations. The IACUC needs to be informed about the dose and route of administration of this chemical and any expected adverse outcomes. The PI needs to provide this information based upon prior personal experience and/or relevant reference literature.

Furthermore, Lee needs to present broader, and in some areas more specific, information describing humane endpoints. Although listing some signs of pain and distress in the rabbit is a good start, the list is incomplete. Ideally, the protocol should state that intervention will be taken if pain or distress is indicated, as defined by specific examples of signs that will be monitored. By defining pain and distress in general and specific terms, it allows both performance standards and specific criteria to be used in the evaluation of the status of the animal. Examples that should be added to the narrative include: decreased appetite, dehydration, twitching, hunched posture, self-mutilation, increased or decreased temperature, and anorexia, among others. Decreased fecal output, soft feces or night stool, or lack of feces are also often first signs of pain and distress in rabbits. The IACUC needs to ask the investigator whether weight loss is a good indicator of pain or distress in this model, as it could be masked by the additional weight of growing tumors or ascites. If weight loss is an appropriate indicator, then in order to avoid potential disagreements or misinterpretations, a specific percentage should be provided as a weight-loss endpoint. Increased heart rate is not reliable or practical in this model and should be removed from the list.

Regarding the treatment/surgical phase of the protocol, the investigator should be advised to consult the institutional veterinarian for the most appropriate analgesics to use for this procedure. Buprenorphine or other nonsteroidal anti-inflammatory drugs (NSAIDs) are more effective than aspirin. Additionally, pre-emptive analgesics should be administered in order to get pain relief on-board during the recovery phase. Although the investigator attempts to address general indicators of pain, means to recognize localized pain at the incision site need to be included in the protocol narrative. Signs of redness, swelling, dehiscence, and infection are all indications that

veterinary intervention is necessary.

Lastly, the protocol should state the qualifications of those working with the rabbits, specifically addressing their experience in surgery and in identifying pain in rabbits. Poor surgical techniques can lead to prolonged healing times, infection, and unnecessary pain. Experience administering anesthetics to rabbits is also crucial. Depending on the extent of tumor development, the animals' health and tolerance of anesthesia may be compromised.

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RESPONSE

Monitor matters

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Several questions should be considered. First, during anesthesia, what parameters will be monitored? We recommend that at a minimum, respiratory rate, heart rate, temperature, corneal/pedal reflexes, blood pressure, and ECG be monitored.

Second, are the methods to provide relief of pain and distress adequate? In this case, perhaps not; aspirin/NSAIDs are minimally effective against surgical pain, and aspirin may be unpalatable in rabbits. Ideally, pre-emptive opioids with an NSAID (e.g., buprenorphine combined with carprofen, meloxicam, or ketoprofen) should be administered as recommended by the American College of Laboratory Animal Medicine (ACLAM)¹.

Third, who should monitor the rabbits, how should the rabbits be monitored, and at what frequency? According to the American Psychological Association's *Guidelines for Ethical Conduct in the Care and Use of Animals*, "the psychologist should monitor the research and the animals' welfare throughout the course of an investigation to ensure continued justification for the research²." The IACUC and PI should also investigate other related questions: is it prudent to rely on the animal care staff to monitor these rabbits during tumor induction?